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Review

Mitochondrial fatty acid synthesis and respiration

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ABSTRACT

Recent studies have revealed that mitochondria are able to synthesize fatty acids in a malonyl-CoA/acyl carrier protein (ACP)-dependent manner. This pathway resembles bacterial fatty acid synthesis (FAS) type II, which uses discrete, nuclearly encoded proteins. Experimental evidence, obtained mainly through using yeast as a model system, indicates that this pathway is essential for mitochondrial respiratory function. Curiously, the deficiency in mitochondrial FAS cannot be complemented by inclusion of fatty acids in the culture medium or by products of the cytosolic FAS complex. Defects in mitochondrial FAS in yeast result in the inability to grow on nonfermentable carbon sources, the loss of mitochondrial cytochromes *a*/*a3* and *b*, mitochondrial RNA processing defects, and loss of cellular lipoic acid. Eukaryotic FAS II generates octanoyl-ACP, a substrate for mitochondrial lipoic acid synthase. Endogenous lipoic acid synthesis challenges the hypothesis that lipoic acid can be provided as an exogenously supplied vitamin. Purified eukaryotic FAS II enzymes are catalytically active *in vitro* using substrates with an acyl chain length of up to 16 carbon atoms. However, with the exception of 3-hydroxymyristoyl-ACP, a component of respiratory complex I in higher eukaryotes, the fate of long-chain fatty acids synthesized by the mitochondrial FAS pathway remains an enigma. The linkage of FAS II genes to published animal models for human disease supports the hypothesis that mitochondrial FAS dysfunction leads to the development of disorders in mammals.

1. Introduction

Recent research has brought to light many previously unknown or underappreciated areas of physiology and biochemistry of mitochondria. These observations include aspects of mitochondrial fusion and fission events, linkage of mitochondrial fusion events to the progression of the cell cycle, mitochondrial–nuclear crosstalk, mitochondrial DNA replication, transcription and translation, iron–sulfur cluster biogenesis, and the role of mitochondria in apoptosis [1–5]. Among the recently recognized features of mitochondrial functions is their ability to synthesize fatty acids in an acyl carrier protein (ACP)-dependent manner. The dual localization of fatty acid synthesis (FAS) in eukaryotic cells raises the question of why eukaryotes have maintained FAS in the mitochondria in addition to the "classic" cytoplasmic FAS (FAS I). The mitochondrial FAS pathway is composed of a set of monofunctional enzymes resembling the bacterial FAS II system and contrasts with the eukaryotic cytosolic multifunctional complex.

Currently, most of our knowledge of mitochondrial FAS II (mtFAS II) is based on data obtained from experiments with yeast. Yeast cells deficient in mtFAS II have small mitochondria and a respiratory-deficient phenotype, lose spectrally detectable cytochromes, display low lipoate content and have defects in mitochondrial RNA processing (reviewed in Hiltunen et al. [6]). Apart from the role as a substrate in lipoic acid synthesis, the fate of products of mtFAS II is still largely an enigma (Table 1). All the enzymes that have been tested *in vitro* display enzymatic activities with acyl substrates having chain lengths up to 16 carbon atoms. Generation of long-chain fatty acids can be demonstrated also in labeling experiments with isolated mitochondria or their extracts. Disruption of mitochondrial mtFAS II affects the mitochondrial phospholipid composition, but it is unclear whether this is an indirect effect due to disturbed mitochondrial metabolism or a direct consequence of reduced channeling of mitochondrially synthesized acyl groups into phospholipids. Alternatively or additionally, mitochondrially synthesized long-chain fatty acids may be used for protein acylation. A special example is 3-hydroxymyristoyl-ACP, which is a component of mammalian mitochondrial complex I [7]. The best-characterized physiological function of mtFAS II is to provide the octanoyl chain for lipoic acid synthesis. Mitochondrial lipoic acid synthesis seems to be an essential process in mammals; deletion of lipoic acid synthase results in embryonic lethality in mice [8]. The enzymology of mtFAS II has been reviewed recently [9], and thus, this review emphasizes the biology and physiology of this mitochondrial pathway.

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2. Comments on enzymology and substrates of mtFAS II

Most of the proteins of the mtFAS II pathway have been identified using bioinformatic tools. Sequences of bacterial proteins were used as probes to search for homologs, and subsequently, the activity of the candidates was tested by functional complementation *in vivo* and/or assaying recombinant proteins for enzyme activities *in vitro*. As discussed below, however, three enzymes, 3-ketoacyl reductase (KAR) in mammals, 3-hydroxyacyl thioester dehydratase 2 (HTD2) in yeast and mammals, and the yeast 2-enoyl thioester reductase (Etr1p) (Fig. 1), were not found by using this approach.

Whether as a part of multifunctional FAS I complexes or separate entities of FAS II, all known KAR enzymes have similar sequences and belong to the large superfamily of short-chain dehydrogenases/reductases. Although the KAR enzymes share sequence similarity, two mammalian homologs (provided with yeast mitochondrial targeting sequences) only weakly complemented the yeast *oar1*Δ mutation. Based on the hypothesis that both gene products were required simultaneously to replace the missing function, the *oar1*Δ yeast cells were transformed with both homologs on separate yeast expression plasmids. The result of this experiment indicated that mammalian KAR is comprised of 17β-hydroxysteroid dehydrogenase type 8 (17β-HSB8) and a carbonyl reductase type 4 (CBR4). When the two components were expressed together, they rescued the respiratory-deficient phenotype and restored the lipoic acid content of the *oar1Δ* yeast strain to wild-type levels. 17β-HSB8 and CBR4 form a mitochondrial complex, which can be copurified as a heterotetrameric ($\alpha_2\beta_2$) assembly when coexpressed in *Escherichia coli*. The heterodimeric nature of human KAR is novel and unexpected and explains its resistance to identification via bioinformatics. Most of the known KARs catalyze the reduction of 3-ketoacyl thioesters in an NADPH-dependent manner, with the exception of the mammalian enzyme [10] and the enzyme from *Caenorhabditis elegans*, which are both NADH-dependent [11].

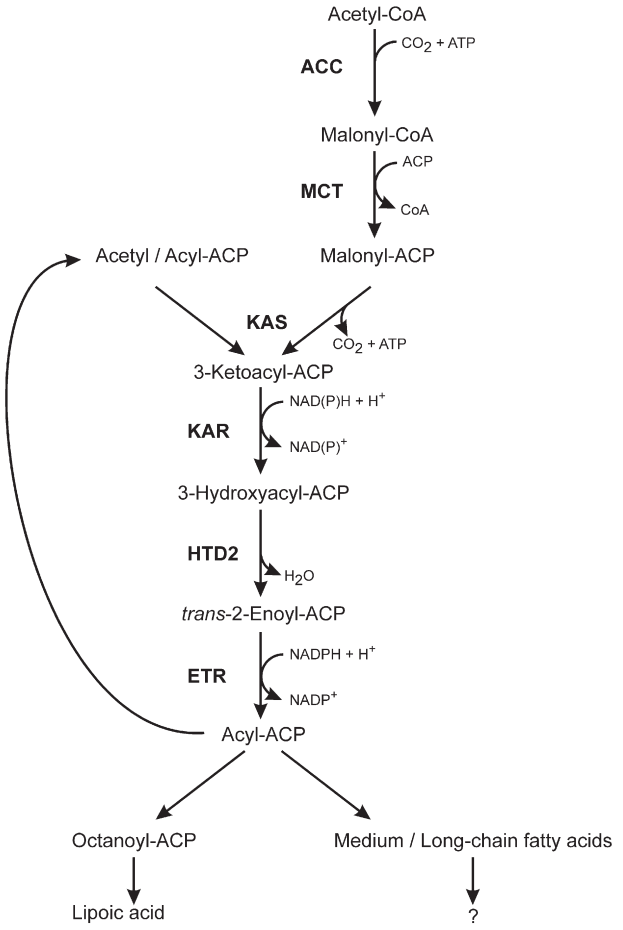

Fig. 1. Mitochondrial fatty acid synthesis pathway. Schematic presentation of the sequence of events. The indicated abbreviations follow the nomenclature used for the human enzymes and proteins. ACC, acetyl-CoA carboxylase; MCT, malonyl-CoA transferase; ACP, acyl-carrier protein; KAS, ketoacyl synthase; KAR, ketoacyl reductase; HTD2, hydroxyacyl-thioester reductase type 2; ETR, enoyl-thioester reductase.

The FAS dehydratases from *E. coli* do not have clear homologs in *Saccharomyces cerevisiae*, and an attempt to purify the enzyme from yeast extracts failed. However, a genetic approach led to the identification of yeast mutants, which could be complemented by a mitochondrially targeted chimeric bacterial hydroxyacyl-ACP dehydratase encoded by *fabA*. In this screen, mutants were identified which were unable to lose a plasmid carrying this construct when grown on glycerol, a nonfermentable carbon source. *YHR067w* (later named *HTD2)* was identified as the gene carrying the mutation [12]. The *htd2* mutant yeast cells exhibited a respiratory-deficient phenotype, loss of mitochondrially encoded cytochromes, and a low level of lipoic acid, similar to other mitochondrial FAS mutants. Surprisingly, no functional mammalian homolog of yeast Htd2p could be found in the genome databases. Screening of human cDNA libraries for clones that rescued respiratory deficiency of the *htd2Δ* yeast strain identified several candidates. Surprisingly, all of the rescued library plasmids harbored *RPP14* cDNA, previously described to encode the RPP14 subunit of human RNase P. When we further examined the cDNA, we found that it carried two open reading frames on the same transcript. We were able to demonstrate by genetic and biochemical means that the open reading frame 3′ of the previously characterized RPP14 sequence encoded human 3-hydroxyacyl thioester dehydratase 2 (HTD2) [13]. Both the fungal and mammalian mitochondrial FAS dehydratases are members of the MaoC dehydratase-like subfamily of thioester/thiol ester dehydratase/isomerase (TED1) superfamily.

The last reaction of the mtFAS II cycle is catalyzed by Etr1-type enoyl thioester reductases. The first eukaryotic Etr1 was identified by purifying and subsequently cloning the gene for an enzyme capable of reducing double bonds in 2,4-hexedienoyl-CoA in an NADPH-dependent manner from *Candida tropicalis* [14]. This protein was shown to be a 2-enoyl-CoA/ACP reductase of mtFAS II, and because it accepts both CoA and ACP thioesters as substrates, it was named enoyl thioester reductase. The enzymes involved in fatty acid synthesis in various subcellular compartments and organisms comprise a fascinating example of functional convergence during evolution. Namely, most of the prokaryotic enzymes are short-chain alcohol dehydrogenase proteins. In contrast, eukaryotic mitochondrial Etr1s (the enoyl reductases of the fatty acid chain elongation system in the endoplasmic reticulum and the corresponding catalytic domain in the mammalian cytosolic FAS complex) are medium chain alcohol reductase/dehydrogenases [14–16]. Curiously, the enoyl reductase domain of yeast cytosolic FAS and bacterial FabK both have a TIM-barrel fold [16].

3. Lipoic acid and protein lipoylation

Although almost 60 years have passed since the first identification of lipoic acid (thioctic acid, thiotic acid, 6,8 dithiooctanoic acid) [17], the enzymatic steps that lead to the synthesis of lipoic acid (LA) and protein lipoylation in *E. coli* have been characterized in detail only recently [18–23]. Several aspects of the generation of this important mitochondrial enzyme cofactor are still not well understood. Moreover, the investigation of eukaryotic LA synthesis has begun only recently.

LA acts as a “swinging arm” domain in keto acid dehydrogenases, which are enzyme complexes that carry out the oxidative decarboxylation of 2-keto-acids [24]. Typically, these complexes are composed of oligomeric superstructures of three subunits, a decarboxylase (E1, mono/dimeric), a dihydrolipoyltransferase (E2), and the dihydrolipoyl dehydrogenase (E3). Yeast has two of these enzyme complexes, α-ketoglutarate dehydrogenase (KDH) and pyruvate dehydrogenase (PDH), while higher eukaryotes also harbor a mitochondrial branched chain α-

keto acid dehydrogenase (BCD). PDH also contains a fourth protein (protein X, Pdx1p in yeast) homologous to E2, which is required for binding of E3 to E2 in yeast (Table 1) [25]. An additional lipoylation-dependent complex is the glycine cleavage system/glycine decarboxylase (GC) [26]. This multiprotein complex comprises a decarboxylase (P), a dihydrolipoyl dehydrogenase (L) identical to the E3 subunits, tetrahydrofolate dehydrogenase (T), and the loosely associated LA-bearing H protein. While not quite as highly similar to the keto-acid dehydrogenases as they are to each other, GC is also related to these enzymes. All these complexes are mitochondrial in eukaryotes, and in all cases, LA is attached via an amide bond to the N6 amino group of a conserved lysine residue in the E2 subunit or the analogous H protein of GC.

4. Lipoic acid synthesis

LA metabolism has been investigated most thoroughly in *E. coli*. In this model organism, *de novo* synthesis of the cofactor starts from octanoic acid moieties produced by the bacterial FAS II pathway. Experimental evidence supports a model of transfer of octanoic acid from octanoyl-ACP by lipoyl-(octanoyl)-protein ligase (LipB) to the target proteins before sulfur insertion by lipoic acid synthase (LipA) [19], an enzyme with similarity to biotin synthase [27]. The sulfur insertion by the dual iron–sulfur cluster enzyme LipA requires two molecules of *S*-adenosyl-L-methionine per molecule of LA produced [28,29].

E. coli can also utilize lipoic or octanoic acid supplied exogenously in an ATP-dependent transfer route catalyzed by the lipoyl-(octanoyl-) protein ligase (LplA) [30,31]. The transfer process occurs in two distinct steps. In the first reaction, LA is activated to lipoyl-AMP, while the second reaction results in the transfer of LA to the recipient protein [30]. An intriguing question is whether LA is available in its free form under physiological conditions. *E. coli* apparently lacks an enzyme with lipoamidase function that is required for release of the cofactor from lipoylated oligopeptides or proteins [32]. Most LA is found bound to proteins via a stable amide linkage and is released only by harsh treatment (prolonged acid hydrolysis under pressure and high temperature), conditions not available in the mammalian gut. It has been reported, however, that cell sap from plant leaves contains a low but measurable amount of free LA [33].

In eukaryotes, several homologs of the *E. coli* enzymes have been identified (Table 2). Eukaryotic lipoic acid synthase was first identified in yeast. The gene was cloned by complementation of a mutation causing respiratory deficiency as well as, curiously, a mitochondrial tRNA processing defect [34]. Further analysis of the mutant strain demonstrated that a mutation in the *LIP5* gene, encoding a protein with high similarity to *E. coli* LipA, was responsible for the phenotype. Growth of the *lip5* mutant on media supplemented with LA did not rescue respiratory deficiency. It was shown later that lipoylated proteins cannot be detected in a *lip5*Δ yeast strain [35]. A mouse homolog of *LIP5* and *lipA* was identified in 2001 [36]. Inactivation of the mouse gene (*Lias*) led to embryonic lethality, and this phenotype could not be rescued by administration of LA to the mothers [8]. From these studies, it is clear that lipoic acid must be synthesized intramitochondrially to support normal growth and development.

Table 2
Lipoic acid synthesis and transfer enzymes.

Function/Organism	Gene	Reference
Lipoic acid synthase		
E. coli	*lipA*	[27]
S. cerevisiae	*LIP5*	[34]
Mammalian	*Lias* (mouse), *LIAS* (human)	[36,50]
Lipoyl-(octanoyl)-protein ligase		
E. coli	*lipB*	[82]
S. cerevisiae	*LIP2*	[37]
Mammalian	*LIPT2*	[83,84]
Lipoyl-(octanoyl)-protein ligase		
E. coli	*lplA*	[30,31]
S. cerevisiae	*LIP3*	[35]
Mammalian	*LIPT1*	[38,39]
Lipoate-activating enzyme		
Mammalian	*ACSM1*	[40]

Yeast lipoyl-(octanoyl)-protein ligase (Lip2p) [37] is required for respiration and is sufficient for the transfer of octanoic acid/LA to Gcv3p, the H protein of GC (Fig. 2). It is also necessary for the lipoylation of the E2 subunits of KDH and PDH but is unable to support the modification of the latter two proteins in the absence of Lip3p, the yeast homolog of bacterial LplA [35] (see below). To date, mammalian homologs of Lip2p/LipB have been identified but not characterized.

Human lipoyltransferase, LIPT1, shares 31% identity with *E. coli* LplA [38]. However, like the bovine homolog [39], LIPT1 lacks the ability to activate LA. It has been shown that in cows, this first step required for attachment of free LA to proteins may be carried out by a separate protein called lipoate-activating enzyme (ACSM1 = acyl-CoA synthetase medium chain family member 1), which requires GTP instead of ATP to form lipoyl-GMP [40]. However, as the gene name suggests, this protein also has high medium chain acyl-CoA synthetase activity and may also play a role in mitochondrial β-oxidation [41]. The yeast homolog encoded by the ORF *YJL046w* was initially identified in a screen for mutants deficient in mitochondrial RNA processing [35]. Sequence analysis indicated similarity to lipoyl transferases, and subsequent tests indicated that the *yjl064w*Δ strain was unable to complement the previously described LA-deficient *lip3* mutation reported in a collection of respiratory-deficient yeast strains [42], and consequently, the gene was termed *LIP3* [35]. It was demonstrated by Western blot analysis using anti-LA antiserum that *LIP3* was necessary but not sufficient for the lipoylation of the Lat1p and Kgd2p E2 subunits of PDH and KDH, respectively. Intriguingly, however, despite very low cellular LA levels, the Gcv3p H protein was lipoylated in the *lip3*Δ mutant. Moreover, deletion of *GCV3*, but not of the other components of GC, completely abolished protein lipoylation in yeast [35]. It was postulated from these results that Lip2p, Lip3p, Lip5p, and Gcv3p may form a lipoylation complex. The E3/L protein, Lpd1p, also may be required for efficient lipoylation, as deletion of the *LPD1* gene results in a reduction of lipoylated proteins in yeast cells. In a "Faculty of 1000" comment on this

Table 1
Confirmed and proposed products and functions of mtFAS II.

Product	Function	Model system	Reference
Lipoic acid	Cofactor for pyruvate dehydrogenase,	Pea leaves	[75]
	α-ketoglutarate dehydrogenase,	Yeast	[57,76]
	the branched chain α-keto acid dehydrogenase and glycine cleavage complexes	Bovine heart	[77]
3-Hydroxymyristoyl-ACP	Component of complex I	Bovine heart	[7]
Long-chain fatty acids	[a]Phospholipid remodeling	Yeast	[78]
		T. brucei	[79]
	[a]Protein acylation/membrane anchor	Yeast	[80,81]

[a] Suggested.

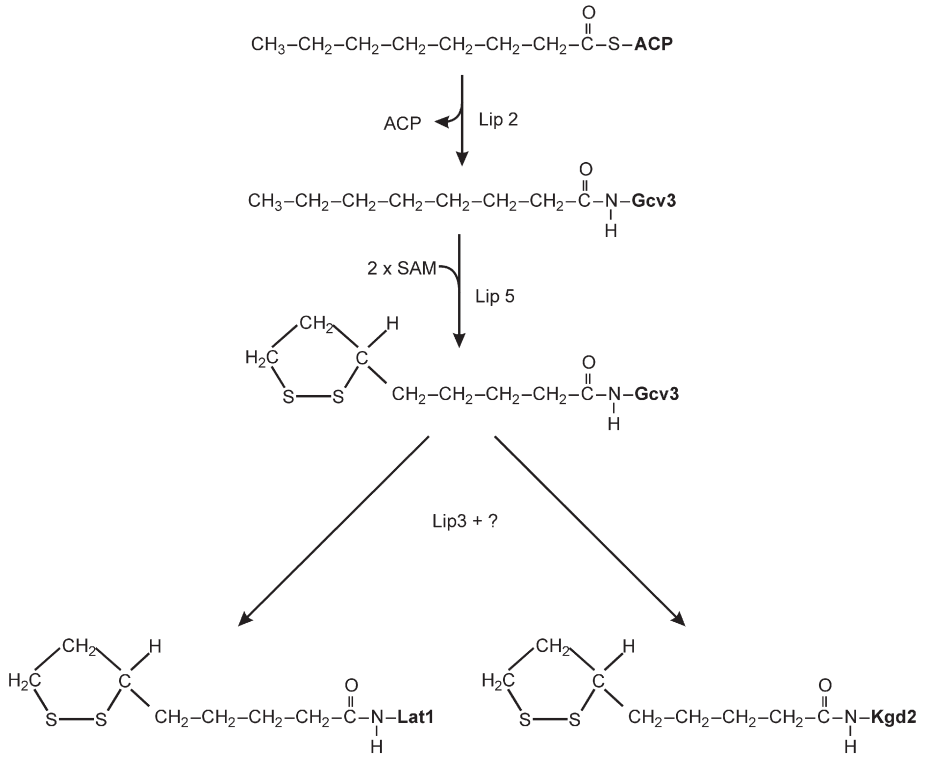

Fig. 2. Model proposed for lipoic acid synthesis and attachment in yeast. According to the model, Lip2 transfers an octanoyl group from ACP to Gcv3p (the H-protein) of the glycine cleavage system followed by lipoic acid synthesis in the reaction catalyzed by Lip5p. Lipoylated Gcv3p acts as a substrate intermediate for lipoylation of other mitochondrial target proteins in a Lip3p-dependent manner. The question mark indicates that the details of this process are unknown. For details, see text. SAM, *S*-adenosyl-L-methionine.

article, Squire J. Booker suggested that Gcv3p, modified by Lip2p, may be a lipoylation intermediate acting as a substrate for Lat1p and Kgd2p lipoylation (Fig.2). While this is a tempting speculation, it has to be pointed out that the data available thus far are not sufficient to draw this conclusion, as direct transfer of LA from Gcv3p to Lat1p or Kgd2p remains to be demonstrated. It also requires the postulation of a novel lipoamidase transferase activity, which has not been described to date. An alternative mechanism with two or three separate activities for cleavage of LA from Gcv3p, activation of LA and transfer to the other targets appears unnecessarily complicated, but cannot be ruled out. Can Lip3p transfer LA directly from Gcv3p to the E2 proteins of KDH and PDH? How would this be achieved on the molecular level? It will be interesting also to determine if lipoylation in humans follows a similar route. It is not clear why lipoylation of Gcv3p in yeast mitochondria is a prerequisite for lipoylation of the other enzymes. A Gcv3p intermediate certainly adds an extra layer of control to the lipoylation process, possibly coordinating mitochondrial one-carbon metabolism with respiration.

The only lipoamidase characterized thus far is Lpa of *Enterococcus faecalis*, an organism unable to synthesize LA and condemned to scavenging LA from its environment [32]. Lipoamidase activities have been reported to have been isolated from pig brain [43] and other mammalian sources as well as yeast [44], but the existence of these enzymes is controversial [32], and an additional transferase activity has not been reported. However, it was demonstrated in ACP knockdown experiments in HEK293 cell culture that levels of LA-modified proteins are undetectable with the LA-specific antiserum, but the levels of the unmodified proteins are unchanged. Hence, LA appears to be turned over more rapidly than the proteins in mammalian cells, implying the existence of an activity for removal of this moiety [45].

5. Endogenously synthesized versus alimentary-acquired lipoic acid

The discovery of the mammalian mtFAS II pathway and lipoic acid synthase challenges the hypothesis that lipoic acid is a vitamin that can be supplied exogenously. While there have been many studies on uptake and metabolism of free LA from industrially synthesized sources (e.g., [46–48]), to our knowledge there is no comprehensive investigation on the bioavailability of LA from natural foodstuffs, and the fate of externally supplied LA in mammals has not been properly addressed. Likewise, while it is obvious that the main features of LA resulting in beneficial effects are its role as an antioxidant and as an inducer of endogenous antioxidants [48], it is not clear if dietary-supplied LA is utilized as a cofactor in mitochondrial enzymes complexes. In yeast, LA added to the growth medium does not rescue the respiratory deficiency of a *lip5* lipoic acid synthase mutant [34]. It is stated in this work that LA could be recovered in protein-bound form, but it was not shown to which proteins and by what linkage LA was attached. It was also concluded by others that exogenously supplied LA is either not taken up into yeast mitochondria or not activated [49], and our own unpublished work supports this conclusion. The embryonic lethality of the mouse *Lias* knockout mutant was not rescued by addition of LA to the diet of the mother [8], and supplementation of ACP knockdown HEK293 cells with LA also had no effect on the lipoylation phenotype of these cells, indicating that exogenously supplied LA may not be available also to the mammalian mitochondrial enzymes [45]. On the other hand, a beneficial effect of lipoic acid on lipoic acid synthase knockdown in cell culture was reported [50]. Also, addition of LA to fibroblasts from a patient with multiple deficiencies in LA-dependent enzyme activities apparently improved BCD function [51], but it was not shown if this effect was caused by incorporation of the cofactor into BCD, or a general improvement of mitochondrial function due to the antioxidant properties of LA. It is remarkable, however, that the latter publication may actually describe a patient suffering from a genuine LA metabolism or mtFAS II deficiency. Interestingly, another group of patients displaying a similar syndrome affecting multiple mitochondrial functions was described more recently [52]. Although the PDH E1 subunit activity defect of these patients may indicate a thiamine deficiency rather than mtFAS II or LA synthesis lesions, patients with defects in the latter two linked pathways may display a similar pattern of symptoms.

6. Mitochondrial RNA processing and mtFAS II in yeast

Both human and yeast mitochondrial genomes are transcribed into multigenic precursor RNAs that must be processed to produce mature messenger, transfer, and ribosomal RNAs. The major precursor RNA produced in mitochondria of most eukaryotes and many fungi is almost of genome-length; the majority of the genes are coded for on one strand of the DNA (reviewed in Falkenberg et al. [53]). mRNAs and rRNAs are interdigitated with tRNAs [54] and thus are freed from the precursor by the enzymes that process the 5′ and 3′ ends of tRNAs, RNase P, and an unidentified 3′ tRNA endonuclease, respectively. The *S. cerevisiae* mitochondrial genome is expanded in size by large regions of noncoding AT-rich sequences and introns, and several major precursor RNAs contain combinations of mRNAs, rRNAs and tRNAs (reviewed in Dieckmann and Staples [55]). The presence of introns and the uneven distribution of tRNAs are such that processing of the precursors to functional mature RNAs requires more than just the tRNA processing enzymes (reviewed in Schafer [56]).

In the pursuit of unidentified RNA processing enzymes in yeast mitochondria, we undertook a screen of the haploid yeast gene knockout collection for uncharacterized genes defective in the processing of complex precursor RNAs. Two deletion mutants were identified that had a retarded rate of processing of the precursor RNA containing both the prolyl tRNA and the RNase P RNA. Quite surprisingly, the mutant with the most severe phenotype had a deletion in a gene coding for an enzyme of the fatty acid biosynthetic pathway exclusive to the organelle, mtFAS II. Subsequently, it was found that deletions of any of the genes coding for the mtFAS II enzymes resulted in a similar phenotype [57]. The other mutant discovered in the screen had a less severe backup of precursor RNA and was missing the yeast homolog of an enzyme in the salvage pathway for lipoic acid attachment in *E. coli* [35].

As shown in Fig. 3, the processing event that is retarded in the mutant strains is an RNase P cleavage at the 5′ end of $tRNA^{pro}$. It is curious that the activity of RNase P is required for processing the precursor RNA that contains the RNase P RNA (RPM1). The RNA assembles with a protein, Rpm2p, which is encoded in the nucleus, translated in the cytoplasm, and imported into the organelle [58]. The requirement of RNase P activity for the maturation of its own RPM1 RNA subunit creates a positive feedback loop that may make the induction/turndown of mitochondrial function more switch-like in response to nutritional or other cues [59].

Since lipoic acid is a key synthetic product of the mtFAS II–LAS pathway, it was attractive to hypothesize that Rpm2p might be modified by lipoic acid. However, we cannot detect lipoic acid modification of Rpm2p using an antibody against lipoic acid [57], which detects the cofactor cleanly on the abundant E2 subunits of PDH and α-KDH, and the H protein of GC. Moreover, since the RNA processing defect of the mtFAS II mutants is more severe than that of any one of the LAS mutants or combinations thereof, our current hypothesis is that a product of the mtFAS II pathway, octanoic acid or a longer fatty acid, may modify Rpm2p covalently, or affect RNase P activity by noncovalent association, or affect its activity indirectly.

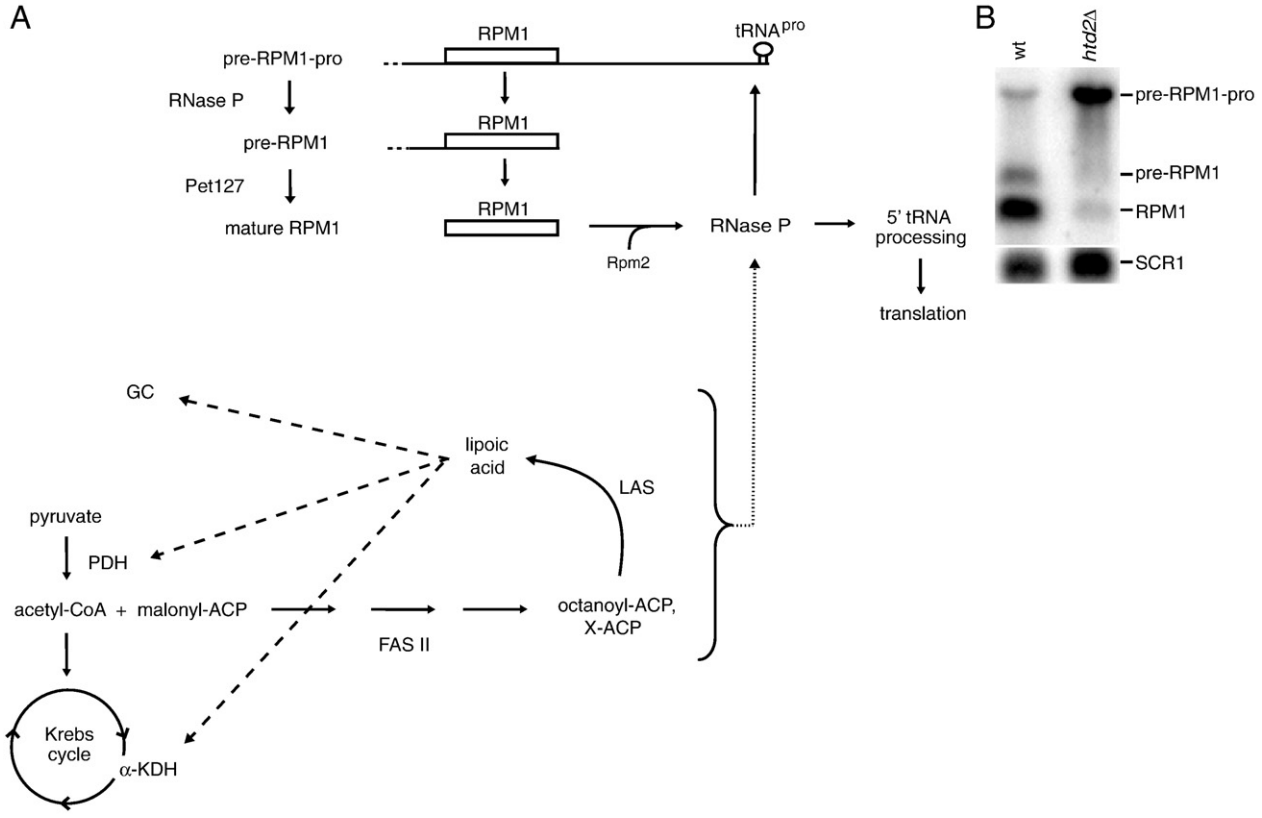

Fig. 3. Intersection of the mtFAS II-LAS pathway and RNase P activity in yeast mitochondria. (A) The major product of the mtFAS II pathway is octanoyl-ACP. Enzymes in the LAS (lipoic acid synthesis) pathway convert octanoic acid to lipoic acid and attach the cofactor to target proteins. The dashed arrows represent the requirement of lipoic acid for GC, PDH and α-KDH enzymatic activity. The dotted arrow represents the requirement of a product of mtFAS II or LAS for efficient processing of *RPM1*-containing transcripts by RNase P. *RPM1* is transcribed in a single transcription unit with downstream $tRNA^{pro}$. RNase P processes the pre-*RPM1-pro* precursor RNA at the 5′ end of $tRNA^{pro}$, generating the pre-*RPM1* intermediate. Further processing at the 5′ end of this intermediate in a Pet127-dependent reaction produces mature *RPM1* RNA. Positive feedback loops for PDH activity and RNase P activity are shown. (B) A Northern blot of total RNA extracted from wild-type and *htd2Δ* strains was hybridized with a probe complementary to *RPM1*. A probe complementary to *SCR1*, the RNA subunit of the cytoplasmic signal recognition particle, was used as a loading control.

7. mtFAS II and RNA processing in mammals?

The mammalian mitochondrial genome exists as a closed circular molecule, with a size of 16,569 bp in humans [60] and approximately 16,300 bp in mouse [61]; thus, it is relatively small compared to yeast mitochondrial DNA. It codes for 13 polypeptides needed for oxidative phosphorylation, as well as 2 ribosomal RNAs and 22 transfer RNAs for protein translation. The transcription of either strand produces polycistronic precursor RNA molecules, which are then further processed to separate mRNAs, tRNAs, and rRNAs. Similar to yeast, genes encoding proteins and rRNAs are bordered by at least one tRNA gene [60], and thus, processing of 5′ ends of tRNAs by RNaseP and 3′ ends by an uncharacterized enzyme leads to separation of all the other RNAs [62].

Nuclear RNase P is responsible for the 5′ processing of all nuclear tRNA precursors before export to the cytoplasm. The yeast enzyme comprises many protein subunits and a nuclearly encoded catalytic RNA. RPP14, one of the nuclear RNase P protein subunits, is translated from a highly conserved bicistronic mRNA, which also encodes mtFAS II dehydratase Htd2p [13]. The rare bicistronic nature of this mRNA hints at a possible link between mtFAS II and RNA processing in vertebrates.

Mitochondrial tRNAs are transcribed as part of larger precursor transcripts and also require 5′ processing by an RNase P activity. It has been thought that the nuclear enzyme is imported into the organelle because it was purified from isolated HeLa cell mitochondria [63]. Also, there is no RNase P RNA encoded in the human mitochondrial genome that would hint at a separate enzyme assembled in the organelle. However, recently, a mitochondrial RNase P containing no RNA subunit, or any protein subunits common to the nuclear enzyme, was purified from HeLa cell mitochondria [64].

Human MRP nuclease, which is required for rRNA processing in the nucleolus [65,66], shares many protein subunits with the nuclear RNase P [67,68], although RPP14 may be underrepresented in this enzyme. MRP nuclease has been reported to be localized in mitochondria also [69], where it cleaves the RNA primers required for mitochondrial DNA replication [70]. Why the RPP14 nuclease subunit and HTD2 dehydratase are expressed from the same mRNA remains a mystery.

8. mtFAS II and mitochondrial morphology

One aspect of mtFAS II that has remained largely neglected is the effect of loss-of-function mutations or overexpression of mtFAS II components on mitochondrial morphology. Mitochondria of yeast cells lacking functional mtFAS II appear small and rudimentary under the electron microscope and exhibit a fragile, more highly branched morphology than mitochondria of wild type cells when examined by fluorescence microscopy [12,14,57]. While it may not be surprising that the nonfunctional organelle also exhibited changes in physical appearance, the phenotype of overexpression mutants is less intuitive. During our studies of yeast Etr1p localization, we observed a remarkable enlargement of the mitochondrial compartment [14] upon overexpression of either the wild-type *S. cerevisiae* or the *C. tropicalis* protein under transcriptional control of the *S. cerevisiae CTA1* catalase promoter. This effect was also observed, albeit to a lesser extent, upon overexpression of mitochondrially localized *E. coli* FabI, the homolog of Etr1p, indicating that the phenomenon is likely due to increased enoyl reductase activity. Likewise, overexpression of Htd2 dehydratase resulted in enlarged mitochondria, but the effect was less dramatic [12]. As observed by electron microscopy, the enlarged organelles appeared to lack or only possess rudimentary cristae structures [12,14]. Recently, we have reported a similar mitochondrial morphology phenotype when the Mecr homolog was overexpressed in mice [71], indicating a possible conservation of function in maintenance or modulation of mitochondrial shape and appearance. Thus far, there is no explanation on a molecular level for these observations.

9. A regulatory role for mtFAS II in the mitochondrial response to pyruvate/acetyl-CoA?

One of the major sources of mitochondrial carbon input is in the form of pyruvate produced by glycolysis. Pyruvate enters the organelle and is converted to acetyl-CoA by the immense pyruvate dehydrogenase complex [72]. The activity of this enzyme requires the swinging arm lipoic acid cofactor to carry the reaction intermediates between the three distinct active sites of the enzyme and participate in the reactions. Most of the acetyl-CoA produced by the enzyme flows into the tricarboxylic acid cycle to produce reducing equivalents for the electron transfer chain. However, some of the acetyl-CoA is the substrate for the mtFAS II pathway. Similar to the positive feedback cycle in mitochondrial RNase P production and activity, there is an additional positive feedback cycle in pyruvate dehydrogenase activity and LA synthesis/attachment in the organelle (Fig. 3). Furthermore, these two positive feedback cycles are linked by the need for an mtFAS II product to optimize RNase P activity. It is tempting to speculate that these cycles exist to regulate mitochondrial function in response to nutritional input.

10. mtFAS II and disease

As previously mentioned in this review, there exist reports of patients with syndromes consistent with a defect in mtFAS II or LA synthesis [51,52] and of cell death in cultured human embryonic kidney 293T cells upon shutdown of ACP using RNAi technology, which compromises protein lipoylation and respiratory complex I activity [45]. There is also other emerging evidence that links the mtFAS II pathway to disease in mammals. Our recent report on the development of cardiomyopathy in mice overexpressing Etr1p established a possible connection between mtFAS II and heart disease (Fig. 3) [71]. Furthermore, decreased expression of *17βHSD8*, a subunit of KAR1 [10], has been associated with disease. *17βHSD8* is localized in the leukocyte antigen centromeric region of chromosome 6, which is a critical disease-susceptibility region affecting multiple pathways [73]. The expression of *17βHSD8* was severely repressed in kidney and liver of polycystic kidney disease mouse models tested thus far [74]. As we learn more about the mitochondrial FAS II and LAS pathways, additional human diseases and syndromes may be linked to deficiencies of the enzymes in these pathways.

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Glossary

The abbreviations for lipoic acid synthesis attachment pathways in different species are summarized in the Table 2.

17βHSD: 17β-hydroxysteroid dehydrogenase
ACP: acyl carrier protein
BCD: branched-chain keto-acid dehydrogenase
E1, E2, and E3: a decarboxylase, dihydrolipoyltransferase, and dihydrolipoyl dehydrogenase subunits in keto-acid dehydrogenase enzyme complexes, respectively
ETR: enoyl thioester reductase
FAS: fatty acid synthesis
GC: glycine cleavage
Gcv3p: the H-protein of glycine cleavage system
HTD: 3-hydroxyacyl thioester dehydratase
KAR: 3-ketoacyl reductase
KDH: α-ketoglutarate dehydrogenase
LA: lipoic acid
Lat1p: dihydrolipoyltransferase (E2) in yeast
mtFAS: mitochondrial FAS
PDH: pyruvate dehydrogenase